Customized FORM PTO-1390 U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

ATTORNEY DOCKET NO.
P07337US00/LRP

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

U.S. APPLICATION NO. (If known, see 37 CFR 1.5)
09/913731

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/EP00/01549	17 FEBRUARY 2000	19 FEBRUARY 1999

TITLE OF INVENTION: METHOD FOR DETERMINING THE SUSCEPTIBILITY OF A NIDDUM PATIENT . . .

APPLICANT(S) FOR DO/EO/US: AMOUYEL, Philippe et al.

Applicant herewith submits to the US Designated/Elected Office (DO/EO/US) the following items and other information:

☒ 1. This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
☐ 2. This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 USC 371.
☒ 3. This express request to begin national examination procedures (35 USC 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 USC 371(b) and PCT Art. 22 and 39(1).
☒ 4. A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
☒ 5. A **copy** of the International Application as filed (35 U.S.C. 371 (c)(2))
  ☐ a. is transmitted herewith (required only if not transmitted by the International Bureau).
  ☒ b. has been transmitted by the International Bureau.
  ☐ c. is not required, as the application was filed in the United States Receiving Office (RO/US).
☐ 6. A **translation** of the International Application into English (35 U.S.C. 371(c)(2)).
☒ 7. Amendments to the claims of the International Appln. under PCT Article 19 (35 USC 371 (c)(3))
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☐ 9. An **oath** or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
☐ 10. A translation of the annexes to the Int'l Prelim. Exam. Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 20. below concern document(s) or information included:

☐ 11. An **Information Disclosure Statement** under 37 C.F.R. 1.97 and 1.98.
☐ 12. An **Assignment** document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
☒ 13. A **First preliminary amendment**.
☐ 14. A Second or Subsequent preliminary amendment.
☐ 15. A substitute specification.
☐ 16. A change of power of attorney and/or address letter.
☐ 17. A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 & 35 USC 1.821-825.
☐ 18. A second copy of the published international application under 35 USC 154(d)(4).
☐ 19. A second copy of the English translation of the international application under 35 USC 154(d)(4).
☐ 20. Other items or information:

☐
☐

☐ A copy of the Notification of Missing Requirements under 35 U.S.C. 371.

☐ In the event that a petition for extension of time is required to be submitted herewith, and in the event that a separate petition does not accompany this response, applicant hereby petitions under 37 CFR 1.136(a) for an extension of time of as many months as are required to render this submission timely. Any fee is authorized in 17(c).

Date: 17 August 2001

LARSON & TAYLOR, PLC • 1199 North Fairfax St. • Suite 900 • Alexandria, VA 22314

JC03 Rec'd PCT/PTO 17 AUG 2001

U.S. APPLICATION NO. (If known) 09/913731	INTERNATIONAL APPLICATION NO. PCT/EP00/01549	ATTORNEY DOCKET NO. P07337US00/LRP

		CALCULATIONS PTO USE ONLY
☒ 21. **The following fees are submitted:**		
☒ **Basic National Fee** (37 CFR 1.492 (a) (1)-(5):		
☐ Neither Int'l Prelim. Exam. fee nor Int'l Search fee paid to USPTO	$1000	
☒ Search Report has been prepared by the EPO or JPO	$ 860	
☐ No Int'l Prelim. Ex. fee paid to USPTO but Int'l Search fee paid to USPTO	$ 710	
☐ International preliminary examination fee paid to USPTPO	$ 690	
☐ Int'l Prelim. Ex. fee paid to USPTO & all claims satisfied PCT Art. 33(1)-(4)	$ 100	
ENTER APPROPRIATE BASIC FEE AMOUNT =		$ 860
☐ Surcharge of **$130** for furnishing the oath or declaration later than ☐ 20 mos. ☐ 30 mos. + from the earliest claimed priority date (37 CFR 1.492(e)).		$

CLAIMS	*NUMBER FILED*	*NUMBER EXTRA*	*RATE*	
Total Claims	11 - 20 =		X **$18** =	$
Independent Claims	02 - 03 =		X **$80** =	$
☐ Multiple Dependent Claim(s) (if applicable)			+ **$270** =	$
TOTAL OF ABOVE CALCULATIONS =				$ 860
☐ Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by ½.			-	$
SUBTOTAL =				$ 860
☐ Processing fee of **$130** for furnishing the **English translation** later than ☐ 20 mos. ☐ 30 mos. + from the earliest claimed priority date (37 CFR 1.492(f)).				$
TOTAL NATIONAL FEE =				$ 860
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TOTAL FEES ENCLOSED =				$ 860
Amount to be				*Refunded* $
				Charged $

☒ a. A **check** in the amount of $ 860 to cover the above fees is enclosed.

☐ b. Please charge my Deposit Account No. 12-0555 in the amount of $ to cover the above fees.

☒ c. The Commissioner is hereby authorized to charge any additional fees required or credit overpayment to Deposit Account No. 12-0555.

Note: *Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.*

SEND ALL CORRESPONDENCE TO:

LINDA R. POTEATE

At the address (below) of **CUSTOMER NO. 00881.**

LARSON & TAYLOR, PLC
1199 NORTH FAIRFAX ST.
SUITE 900
ALEXANDRIA, VA 22314

SIGNATURE: Douglas E Jackson

NAME: Douglas E. Jackson

REG. NO.: 28518

PHONE NO.: 703-739-4900

Date: 17 August 2001

Customized FORM PTO-1390 U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

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☐ Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by ½.			-	$
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SIGNATURE: Douglas E Jackson

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PHONE NO.: 703-739-4900

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09/913731

JC03 Rec'd PCT/PTO 17 AUG 2001

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Patent

In re patent application of: AMOUYEL et al

Serial No.: NEW APPLICATION

Filed: On even date herewith

For: METHOD FOR DETERMINING THE SUSCEPTIBILITY OF A NIDDUM PATIENT . . .

Examiner:

Art Unit:

Docket No.:

P07337US00/DEJ

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C.

S I R:

Prior to examination, please amend the above-identified application as follows.

IN THE CLAIMS:

Please cancel claims 1-12 without prejudice to resubmission. Please add new claims 13-24. A clean version of the new claims is provided herewith in **Attachment A**.

REMARKS

By this Amendment, the claims have been rewritten in order to place the application in better condition for examination.

Further and favorable action is solicited.

Respectfully submitted,

Date: 17 August, 2001

By: [signature]
Douglas E. Jackson
Registration No. 28,518

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Transpotomac Plaza
1199 North Fairfax Street, Suite 900
Alexandria, Virginia 22314
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ATTACHMENT A

New Claims (entire set of pending claims)

Following herewith is a clean copy of the entire set of pending claims.

13. (new) A method for determining the susceptibility of a NIDDM patient toward sulfonylurea therapy comprising :

a) obtaining a sample from a NIDDM patient, said sample comprising nucleic acid molecules containing the fragment of the *SUR1* gene comprising the nucleotide in position –3 of intron 16,

b) detecting the presence or the absence of the –3c allele of intron 16,

whereby the presence of at least one –3c allele identifies a NIDDM patient with a higher susceptibility toward sulfonylurea therapy.

14. (new) The method according to claim 13, further comprising prior to step b) the step of amplifying said nucleic acid molecules using amplification primers that selectively anneal to and amplify a portion of said gene comprising the nucleotide in position –3 of intron 16.

15. (new) The method according to claim 13, further comprising prior to step b) the step of amplifying said nucleic acid molecules using as amplification primers, the nucleic acid fragments of sequence SEQ ID N° 2 and SEQ ID n° 3, that selectively anneal to and amplify a portion of said gene comprising the nucleotide in position –3 of intron 16.

16. (new) The method of claim 13, wherein said detecting step b) comprises sequencing all or part of the sequence of intron 16 comprising said –3 nucleotide.

17. (new) The method of claim 13, wherein said detecting step b) comprises contacting the nucleic acid molecules with a nucleic acid probe that selectively hybridizes to a portion of said 16 intron of *SUR1* gene containing nucleotide -3 as shown in sequence SEQ ID n° 1 under hybridization conditions.

18.(new) The method of claim 13, wherein the detecting step b) comprises performing a restriction endonuclease digestion of said nucleic acid molecules thereby yielding a nucleic acid digest and contacting the digest with a nucleic acid probe that selectively hybridizes to a portion of said intron 16 of said *SUR 1* gene combining nucleotide –3 as showed in sequence SEQ ID n° 1.

19. (new) The method of claim 13, wherein said detecting step b) comprises obtaining a first gene fragment comprising nucleotide –3 of intron 16 isolated from said human sample and a second gene fragment comprising nucleotide –3t of intron 16, said second fragment corresponding to said first fragment, forming single-stranded DNA from said *SUR1* gene fragment and from said second *SUR1* gene fragment, electrophoresing said single-stranded DNAs on a denaturating polyacrylamide gel, comparing the mobility of said single-stranded DNAs on said gel to determine if said single-stranded DNA from said first *SUR1* gene fragment is shifted relative to said second *SUR1* gene fragment, and optionally sequencing said single-stranded DNA from said first *SUR1* gene fragment having a shift in mobility.

20. (new) The method of claim 13 wherein said detecting step b) comprises obtaining a first gene fragment comprising nucleotide –3 of intron 16, isolated from said human sample and a second fragment comprising nucleotide –3c of intron 16, said second fragment corresponding to said first fragment, forming single-stranded DNA from said *SUR1* gene fragment and from said second *SUR1* gene fragment, electrophoresing said single-stranded DNAs on a denaturating polyacrylamide gel, comparing the mobility of said single-stranded DNAs on said gel to determine if said single-stranded DNA from said first *SUR1* gene fragment has the same mobility as the said second *SUR1* gene fragment, and optionally sequencing said single-stranded DNA from said first *SUR1* gene fragment.

21. (new) The method of claim 13 wherein said detecting step b) comprises amplifying all or part of a *SUR1* gene in said sample using a primer specific for allele –

3c and detecting the presence of an amplified product, whereby the presence of said product indicates the presence of said allele in the sample.

22. (new) A kit for determining the susceptibility of a NIDDM patient toward sulfonylurea therapy comprising a pair of oligonucleotide primers specific for amplifying all or part of the *SUR1* gene comprising nucleotide –3 of intron 16, and instructions relating to detecting the presence of a –3c allele of intron 16 and correlating the presence of a –3c allele with a higher susceptibility toward sulfonylurea therapy.

23. (new) The kit according to claim 22 comprising a restriction enzyme that specifically cuts fragments comprising nucleotide –3c/nucleotide –3t, and reagents able to detect the presence of a cleaved fragment, the presence of a cleaved fragment being indicative of a higher susceptibility toward sulfonylurea therapy (-3c)/a lower susceptibility toward sulfonylurea therapy (-3t).

24. (new) The kit according to claim 22 comprising *Pst I as* restriction enzyme that specifically cuts fragments comprising nucleotide –3c/nucleotide –3t, and reagents able to detect the presence of a cleaved fragment, the presence of a cleaved fragment being indicative of a higher susceptibility toward sulfonylurea therapy (-3c)/a lower susceptibility toward sulfonylurea therapy (-3t).

Method for determining the susceptibility of a NIDDM patient toward sulfonylurea therapy.

The present invention relates to a method of determining the susceptibility of a non-insulin-dependent diabetes mellitus (NIDDM) patient toward a sulfonylurea therapy.

Sulfonylureas are oral hypoglycaemic agents widely used in the treatment of NIDDM. They bind to the high affinity sulfonylurea receptor 1 (SUR1) and stimulate insulin release from pancreatic islet β cells. SUR1 is one of the protein that composes the ATP-sensitive potassium channel I_{KATP}, closed by glucose metabolism in pancreatic β cells and triggering insulin exocytosis. The gene encoding SUR1 is located on chromosome 11p15.1. Mutations in the gene have been found in Familial Persistent Hyperinsulinemic Hypoglycaemia of Infancy (PHHI) (Thomas et al, (1995) ; Thomas et al (1986), Kane et al (1996) and Dunne et al (1997)) also known as Familial Hypersinsulinism (HI) (Nestorowicz et al (1996)). This disease is characterized by the elevation of serum insulin levels and severe hypoglycaemia.

Two case-control studies reported an association between genetic polymorphisms in the *SUR1* gene and NIDDM. (Inoue et al, (1996) Hansen et al (1998)). To estimate the impact of the *SUR1* genetic variability on NIDDM in population, the inventors characterized the genotypes of subjects for the most frequent polymorphism of the *SUR1* gene, a –3t→c mutation located in intron 16, namely in position –3 of the exon 17 splice acceptor site (nucleotide 191 of SEQ ID n° 1) in a large representative sample of the French population aged 35 to 64 years.

As a result, they discovered that among the NIDDM patients, the frequency of the c allele was significantly lower in controls than in NIDDM patients.

In controls, no association was found between the polymorphism and body mass index, waist-to-hip ratio, fasting plasma glucose, fasting plasma insulin and lipid and lipoproteins profile. In NIDDM patients, the c allele was associated with a decrease in plasma triglycerides concentrations. NIDDM patients were stratified in two groups : subjects treated with sulfonylureas and subjects treated without. Decreases in plasma triglycerides and VLDL-cholesterol concentrations were found only in c allele bearers treated with sulfonylureas.

The discovery that sulfonylurea therapy seems to be more efficient on hypertriglyceridemia reduction in NIDDM patients with the *SUR1* intron 16 c allele than in NIDDM patients without, may help to a better targeting of the various therapies available in NIDDM treatment.

The present invention relates to a method for determining the susceptibility of a NIDDM patient toward sulfonylurea therapy comprising :

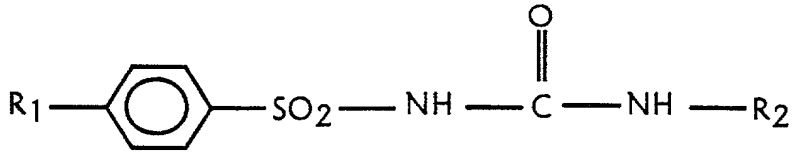
a) obtaining a sample from a NIDDM patient, said sample comprising nucleic acid molecules containing the fragment of the *SUR1* gene comprising the nucleotide in position –3 of intron 16,

b) detecting the presence or the absence of the –3c allele in position –3 of intron 16,

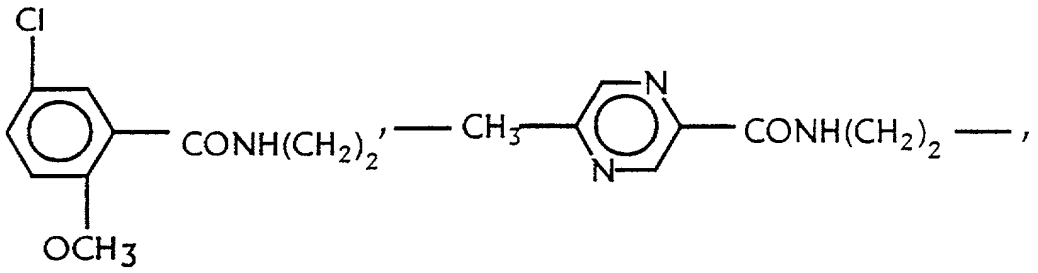
whereby the presence of at least one –3c allele identifies a NIDDM patient with a higher susceptibility toward sulfonylurea therapy.

Sulfonylurea therapy in the sense of the instant invention identifies the current therapies of NIDDM utilizing oral hypoglycaemic agents binding the SUR1 receptor and stimulating insuline release from pancreatic islet β cells.

Such agents are derivatives of arylsulfonylurea having the following general formula :



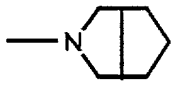
wherein R_1 may have the following meanings : Cl, CH_3,

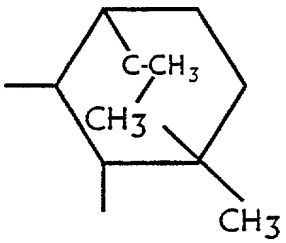


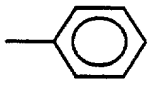
and R_2 may have the following meanings :

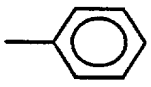
$-(CH_2)_2CH_3$

$-(CH_2)_3CH_3$









The main compounds are known under the following denominations : chlorpropamide, tolbutamide, gliclazide, glibornuride, glibenclamide, glipizide and buformine.

The sample from the patient may be any biological sample containing nucleic acids, namely a blood sample.

Intron 16 of the *SUR1* gene is identified within the instant invention according to the nomenclature of Hansen et al (above) which teaching is hereby incorporated by reference.

The mutation responsible for the polymorphism referred to in the instant invention occurs on nucleotide -3 of the exon 17 splice acceptor site, the first nucleotide of the intron being numbered –1, the second –2 and the third –3 (SEQ ID n° 1, EMBL accession number L78223).

The detection of the –3t→c mutation in intron 16 may be performed by any known method in the art detecting DNA sequence variation.

A review of currently available methods of detecting DNA sequence variation can be found in a review by Grompe (1993).

In a preferred embodiment, the method comprises prior to step b) the step of amplifying said nucleic acid molecules using amplification primers that selectively anneal to and amplify a portion of said gene comprising the nucleotide in position –3 of intron 16.

One method for detecting the –3t→c mutation in the position –3 of intron 16 of the *SUR1* gene comprises sequencing all or part of the sequence of intron 16 comprising said –3 nucleotide.

Direct DNA sequencing, either manual sequencing or automated fluorescent sequencing may be used.

Another approach is the single-stranded conformation polymorphism assay (SSCA') (Orita et al, 1989).

According to that approach, step b) comprises obtaining a first *SUR1* gene fragment comprising the nucleotide in position –3 of intron 16 isolated from a human sample and a second *SUR1* gene fragment comprising nucleotide –3t of intron 16, said second fragment corresponding to said first fragment, forming single-stranded DNA from said first *SUR1* gene fragment and from said second *SUR1* gene fragment, electrophoresing said single-stranded DNAs on a denaturating polyacrylamide gel, comparing the mobility of said single-stranded DNAs on said gel to determine if said single-stranded DNA from said first *SUR1* gene fragment is shifted relative to said second *SUR1* gene fragment.

The fragments which have shifted mobility on SSCA gels are then optionally sequenced to determine the exact nature of the DNA sequence variation.

Alternatively, instead of utilizing as the second gene fragment a gene fragment comprising nucleotide –3t of intron 16, one may utilize a second fragment comprising nucleotide –3c of intron 16, whereby similar mobility is indicative of the presence of the –3t→c mutation in the position –3 of intron 16.

An other approach comprises contacting the nucleic acid molecules with a nucleic acid probe that selectively hybridizes to a portion of said 16 intron of *SUR1* gene containing nucleotide –3 as shown in sequence SEQ ID n° 1 under hybridization conditions.

A further method comprises performing a restriction endonuclease digestion of said nucleic acid molecules thereby yielding a nucleic acid digest and contacting the digest with a nucleic acid probe that selectively hybridizes to a portion of said intron 16 of said *SUR 1* gene containing nucleotide –3 as showed in sequence SEQ ID n° 1.

A still a further method comprises amplifying all or part of the *SUR1* gene in said sample using a primer specific for allele –3c and detecting the presence of an amplified product, whereby the presence of said product indicates the presence of said allele in the sample.

By "higher susceptibility", it is intented that not only hyperglycemia is decreased in NIDDM patients, but also hypertriglyceridemia, which is the main factor of cardiac risk for diabetic patients.

The instant invention also relates to a kit for determining the susceptibility of a NIDDM patient toward sulfonylurea therapy comprising a pair of nucleotide primers specific for amplifying all or part of the *SUR1* gene comprising the nucleotide –3 of intron 16, and instructions relating to detecting the presence or a –3c allele of intron 16 and correlating the presence of a –3c allele with a higher susceptibility toward sulfonylurea therapy.

In a preferred embodiment, the kit comprises a restriction enzyme that specifically cuts fragments comprising nucleotide –3c/nucleotide –3t, and reagents able to detect the presence of a cleaved fragment, the presence of a

cleaved fragment being indicative of a higher susceptibility toward sulfonylurea therapy (-3c)/ a lower susceptibility toward sulfonylurea therapy (-3t).

The restriction enzyme is preferably *Pst I* that cleaves specifically fragments comprising the –3c allele.

The present invention is described by reference to the following examples and the enclosed figure representing the effect of the *SUR 1* intron 16 –3t→c polymorphism on plasma triglycerides concentrations in NIDDM subjects, wherein * indicates that the p value was adjusted for age, gender, body mass index, alcohol and smoking consumptions (linear trend test) and † is indicative that the test used was the non-parametric Wilcoxon test.

Standard techniques well known in the art or the techniques specifically described below were utilized.

Population and methods

Population study

The population study was selected in 1995-1997 from three large representative French samples participating to the risk factor surveys of the WHO-MONICA (Multionational Monitoring of trends and determinants of Cardiovascular diseases) project (Ecological analysis of the association between mortality and major risk factors of cardiovascular disease. The World Health Organization MONICA Project. Int J. Epidemiol. 1994 : 23:505-16 ; Tunstall-Pedoe H et al). This population study was randomly sampled from the electoral rolls of three geographical areas : the Urban Community of Lille (Lille) in the North, the department of Bas-Rhin (Strasbourg) in the East, the department of Haute-Garonne (Toulouse) in the South of France. The number of subjects recruited were 1195, 1131 and 1182 in Lille, Strasbourg and Toulouse areas respectively stratified on ten year age classes and gender. A fasting blood sample was drawn for all participants. In these samples, 123 NIDDM affected individuals were recovered on the basis of a medical diagnosis and on the existence of a specific treatment (Lille n=47, Strasbourg n=41, Toulouse n=35). A control group (n=1250) composed of *individuals* without

diabetes, hypercholesterolemia or hypertension and without any treatment for these diseases was selected.

Biological measurements

Glucose was measured by the glucose oxidase method (DuPont Dimension). Plasma insulin was measured by radio-immunoassay (Bilnsuline, ERIA Pasteur). Serum total cholesterol and triglyceride levels were measured by enzymatic methods (DuPont Dimension).

Genetic analysis

Genomic DNA was extracted from white blood cells as described by Miller, A et al (1988). DNA amplification was performed using Polymerase Chain Reaction (PCR). Typing of the intron 16 –3t→c polymorphism was achieved as described by Inoue et al (above).

Statistics

Statistical analyses were performed with the SAS statistical software, version 6.11 (SAS Institute Inc., Cary, NC). Genotype and allele distributions were compared with Pearson λ^2 statistical tests. The effect of the polymorphism on quantitative variables was tested with a multivariate analysis of covariance using a general linear model (proc GLM, type III SS). Interactions between genotypes and covariates were tested. Data for triglycerides, insulin, and glucose were log transformed to normalize the distributions. Statistical significance was considered at the $p<0.05$ level. When the number of subjects was low, genotypes were compared using non parametric Wilcoxon test.

Results

In control subjects, the frequency of the c allele of the *SUR* intron 16 –3t→c polymorphism was 0.45, 0.44 and 0.50 in Lille, Strasbourg and Toulouse studies respectively. When controls of the three studies were pooled, the frequency of the c allele was 0.46 while it was 0.53 in NIDDM patients. The

adjusted relative risk for *cc* subjects to develop NIDDM was 1.76 (95 % CI : [1.10-2.80], p=0.017) adjusted for age, gender, centre and body mass index.

Possible associations between the intron 16 –3t→c polymorphism and clinical and biological variables such as body mass index (BMI), waist-to-hip ratio, plasma insulin, fasting-plasma glucose. or lipid variables in control and in NIDDM subjects were investigated. In controls, no association was found between the polymorphism and any variables listed above. In NIDDM patients, the -3c allele was associated with decreased plasma triglycerides concentrations (2.03 [1.12-3.71] for *tt*, 1.62 [0.88-2.97] for *tc* and 1.45 [0.85-2.46] mmol/l for *cc*, p=0.023) and in an allele dose dependent manner (Table 2, figure 1).

As the sulfonylurea receptor 1 binds sulfonylurea agents, NIDDM patients were stratified in two groups : NIDDM patients receiving sulfonylureas (n=70) and patients receiving another treatment (n=52). Given the low number of *cc* homozygous subjects, *tc* and *cc* subjects were pooled to analyse a dominant effect of the –3c allele. Wilcoxon tests were performed. In the group treated with sulfonylureas, the intron 16 –3c allele was associated with a statistically significant decrease in plasma triglycerides concentrations (2.20 mmol/l [1.14-4.14] for *tt* versus 1.43 mmol/l [0.81-2.52] for *tc*+ *cc* ; p=0.026) whereas no association was found in the other group (figure 1).

The results indicate that the -3c allele is associated with decreased plasma triglycerides concentrations in NIDDM patients, only in NIDDM patients receiving a sulfonylurea therapy, underlying a pharmacogenetic susceptibility to sulfonylurea treatment response.

This result is in accordance with previous works showing that oral sulfonylurea therapy, in addition to an improvement of glycemic control, decreases hepatic lipase levels and declines the production of triglycerides and VLDL-cholesterol in diabetics (Howard et al (1985) ; Taskinen et al (1986)). The results of the instant invention suggest that sulfonylurea therapy is more efficient on hypertriglyceridemia reduction in NIDDM patients bearing the *SUR1* intron 16 –3c allele underlying a pharmacogenetic susceptibility to sulfonylurea treatment response.

Table 1 : Genotype and allele frequencies of the *SUR* intron 16 3t→c polymorphism in NIDDM patients and control subjects.

	NIDDM patients				Controls
	Lille	Strasbourg	Toulouse	All	
n	47	41	34	122	1250
Genotype frequencies					
tt	9 (0.19)	11 (0.27)	9 (0.27)	29 (0.24)	359 (0.29)
tc	24 (0.51)	18 (0.44)	14 (0.41)	56 (0.46)	620 (0.50)
cc	14 (0.30)	12 (0.29)	11 (0.32)	37 (0.30)*	271 (0.21)
allele frequencies					
t	42 (0.45)	40 (0.49)	32 (0.47)	114 (0.47)	1338 (0.54)
c	52 (0.55)	42 (0.51)	36 (0.53)	130 (0.53)†	1162 (0.46)

Data are n(frequency %). Controls include Lille, Strasbourg, Toulouse studies.

* All NIDDM subjects versus controls *cc/tc+ tt*, p= 0.03.

† All NIDDM subjects versus controls *c/t*, p=0.04.

Table 2 : Effect of the *SUR 1* intron 16-3 t→c polymorphism in NIDDM subjects.

Genotype intron 16	*tt*	*tc*	*cc*	*p*
n	29	55	36	
BMI, kg/m^2	30.7±5.2	29.9±6.2	30.1±6.2	ns
Insulin, µU/ml	20.25 [11.73-34.95]	17.64 [10.28-30.26]	16.78 [8.50-33.11]	ns
Glucose, mmol/l	8.25 [6.05-11.25]	8.25 [6.05-11.25]	8.67 [6.49-11.59]	ns
total cholesterol, mmol/l	5.89±0.94	5.69±0.97	5.61±1.28	ns
Triglycerides, mmol/l	2.03 [1.12-3.71]	1.62 [0.88-2.97]	1.45 [0.85-2.46]	0.023*

* p value was adjusted for age, gender, BMI, alcohol consumption and smoking consumptions (test for linear trend)

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CLAIMS

1. A method for determining the susceptibility of a NIDDM patient toward sulfonylurea therapy comprising :

a) obtaining a sample from a NIDDM patient, said sample comprising nucleic acid molecules containing the fragment of the *SUR1* gene comprising the nucleotide in position –3 of intron 16,

b) detecting the presence or the absence of the –3c allele of intron 16,

whereby the presence of at least one –3c allele identifies a NIDDM patient with a higher susceptibility toward sulfonylurea therapy.

2. A method according to claim 1, further comprising prior to step b) the step of amplifying said nucleic acid molecules using amplification primers that selectively anneal to and amplify a portion of said gene comprising the nucleotide in position –3 of intron 16.

3. A method according to claim 2 wherein said amplification primers are the nucleic acid fragments of sequence SEQ ID N° 2 and SEQ ID n° 3.

4. A method of claim 1, wherein said detecting step b) comprises sequencing all or part of the sequence of intron 16 comprising said –3 nucleotide.

5. The method of claim 1, wherein said detecting step b) comprises contacting the nucleic acid molecules with a nucleic acid probe that selectively hybridizes to a portion of said 16 intron of *SUR1* gene containing nucleotide -3 as shown in sequence SEQ ID n° 1 under hybridization conditions.

6. The method of claim 1, wherein the detecting step b) comprises performing a restriction endonuclease digestion of said nucleic acid molecules thereby yielding a nucleic acid digest and contacting the digest with a nucleic acid probe that selectively hybridizes to a portion of said intron 16 of said *SUR 1* gene combining nucleotide –3 as showed in sequence SEQ ID n° 1.

7. The method of claim 1, wherein said detecting step b) comprises obtaining a first gene fragment comprising nucleotide –3 of intron 16

isolated from said human sample and a second gene fragment comprising nucleotide –3t of intron 16, said second fragment corresponding to said first fragment, forming single-stranded DNA from said *SUR1* gene fragment and from said second *SUR1* gene fragment, electrophoresing said single-stranded DNAs on a denaturating polyacrylamide gel, comparing the mobility of said single-stranded DNAs on said gel to determine if said single-stranded DNA from said first *SUR1* gene fragment is shifted relative to said second *SUR1* gene fragment, and optionally sequencing said single-stranded DNA from said first *SUR1* gene fragment having a shift in mobility.

8. The method of claim 1 wherein said detecting step b) comprises obtaining a first gene fragment comprising nucleotide –3 of intron 16, isolated from said human sample and a second fragment comprising nucleotide –3c of intron 16, said second fragment corresponding to said first fragment, forming single-stranded DNA from said *SUR1* gene fragment and from said second *SUR1* gene fragment, electrophoresing said single-stranded DNAs on a denaturating polyacrylamide gel, comparing the mobility of said single-stranded DNAs on said gel to determine if said single-stranded DNA from said first *SUR1* gene fragment has the same mobility as the said second *SUR1* gene fragment, and optionally sequencing said single-stranded DNA from said first *SUR1* gene fragment.

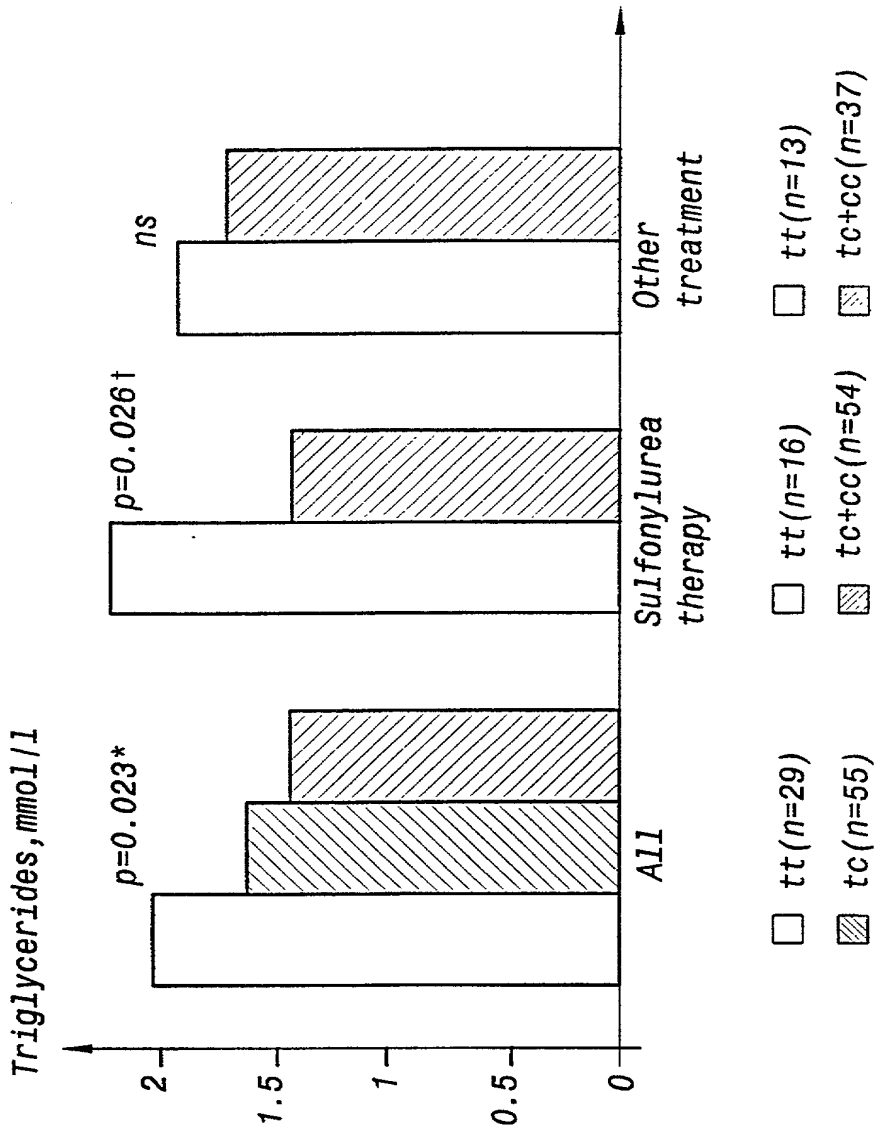
9. The method of claim 1 wherein said detecting step b) comprises amplifying all or part of a *SUR1* gene in said sample using a primer specific for allele –3c and detecting the presence of an amplified product, whereby the presence of said product indicates the presence of said allele in the sample.

10. A kit for determining the susceptibility of a NIDDM patient toward sulfonylurea therapy comprising a pair of oligonucleotide primers specific for amplifying all or part of the *SUR1* gene comprising nucleotide –3 of intron 16, and instructions relating to detecting the presence of a –3c allele of intron 16 and correlating the presence of a –3c allele with a higher susceptibility toward sulfonylurea therapy.

11. A kit according to claim 10 comprising a restriction enzyme that specifically cuts fragments comprising nucleotide –3c/nucleotide –3t, and

reagents able to detect the presence of a cleaved fragment. the presence of a cleaved fragment being indicative of a higher susceptibility toward sulfonylurea therapy (-3c)/a lower susceptibility toward sulfonylurea therapy (-3t).

12. A kit according to claim 11 wherein said enzyme is *Pst I*.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tc+cc(n=54)
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Rec'd PCT/PTO 09/913731

DECLARATION FOR USA PATENT APPLICATION

(including Design and National Stage PCT) Attorney's Docket ID: _____

As a below named inventor, I hereby declare that:
My residence, mailing address and citizenship are as stated below adjacent to my name. I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought

on the invention entitled: Method for determining the susceptibility of a NIDDM patient toward sulfonylurea therapy.

the specification of which:

___ is attached hereto.
(or)
X was filed on February 17, 2000 as U.S. Application No. or PCT International Application No. PCTEP/0001549

and (if applicable) was amended on _____.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment specifically referred to above. I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT International application which designated at least one country other than the United States of America, listed below and have also identified below, where priority is not claimed, any foreign application for patent or inventor's certificate, or any PCT International application, having a filing date before that of the application on which priority is claimed. (___ ADDITIONAL APPLICATIONS IDENTIFIED ON ATTACHED SHEET)

Prior Foreign Application No.	Country	Day/Month/Year Filed	Priority Not Claimed
99400410.9	EUROPE	19.02.1999	___
			___

I hereby claim the benefit under 35 U.S.C. 120 of any U.S. application(s), or 365(c) of any PCT application designating the U.S., listed below; and insofar as the subject matter of each claims of this application is not disclosed in the prior U.S. or PCT application in the manner provided by the first paragraph of 35 U.S.C. 112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56 which became available between the filing date of the prior application and the national or PCT filing date of this application. (___ ADDITIONAL APPLICATIONS IDENTIFIED ON ATTACHED SHEET.)

U.S. or PCT Parent Application No.	Parent Filing Date (Day/Month/Year)	Parent Patent No. (if applicable)
PCT/EP0001549	17.02.2000	

As a named inventor, I hereby appoint the registered practitioners of **LARSON & TAYLOR, PLC** associated with **Customer Number 000881** to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith. Direct all correspondence to that Customer Number.

Direct all telephone calls to _____ at TEL (703) 739-4900 (Fax: 703-739-9577) e-mail:

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true: and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1000 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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SIGN AND DATE HERE Inventor's Signature	Date

SEQUENCE LISTING

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INSERM

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